

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph that begins at page 8, line 16, with the following paragraph:

Accordingly, in certain embodiments, the invention provides mutant apo-B100 proteins and mutant apo-B100 polypeptide fragments, as well as LDL particles and other lipoproteins comprising a mutant apo-B100 protein or polypeptide fragment, which comprise a mutant Site B selected from one of the following Site B sequences:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:1);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:2);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ala**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:3);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Thr**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:4);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ser**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:5);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Gln**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:6);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Glu**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:7);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Asp**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:8);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:9);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:10);

Thr₃₃₅₈-**Glu**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:11);

and

Thr₃₃₅₈-**Asp**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:12);

as well as Site B sequences with deletions, such as:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-----Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:13);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-----Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:14);
and

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-----Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:15);

and Site B sequences which include insertions, such as:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:16);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:17);

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:18);
and

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:19).

Please replace the paragraph that begins at page 17, line 23, with the following paragraph:

The amino acid sequence of the wild-type human apo-B100 protein from amino acid 3358 to 3367 is as follows:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:20).

Please replace the paragraph that begins at page 19, line 1, with the following paragraph:

The following are the amino acid sequences from position 3358 to position 3367 for a list of preferred proteoglycan receptor⁺ apo-B100 protein mutants:

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:1)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:2)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ala**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:3)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Thr**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:4)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Ser**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:5)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Gln**₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:6)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Glu**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:7)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-**Asp**₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:8)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:9)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:10)

Thr₃₃₅₈-**Glu**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:11)

Thr₃₃₅₈-**Asp**₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:12)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-----Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:13)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-----Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:14)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-----Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:15)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Glu**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:16)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Glu**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:17)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-**Asp**-Lys₃₃₆₃-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID NO:18)

Thr₃₃₅₈-Arg₃₃₅₉-Leu₃₃₆₀-Thr₃₃₆₁-Arg₃₃₆₂-Lys₃₃₆₃-**Asp**-Arg₃₃₆₄-Gly₃₃₆₅-Leu₃₃₆₆-Lys₃₃₆₇ (SEQ ID

NO:19), wherein the repeated dashed lines represent deletions.

Please replace the paragraph that begins at page 36, line 13, with the following paragraph:

The polynucleotides of the invention can also be derivatized in various ways, including those appropriate for facilitating transfection and/or gene therapy. The polynucleotides can be derivatized by attaching a nuclear localization signal to it to improve targeted delivery to the nucleus. One well-characterized nuclear localization signal is the heptapeptide PKKKRKV (pro-lys-lys-lys-arg-lys-val) (SEQ ID NO:21). Preferably, in the case of polynucleotides in the form of a closed circle, the nuclear localization signal is attached via a modified loop nucleotide or spacer that forms a branching structure.

Please replace the paragraph that begins at page 38, line 6, with the following paragraph:

Moreover, Borén, J. et al. 1996. *Genome Res.* 6:1123-1130 have demonstrated how to isolate a 5.7 kb fragment of the apo-B100 gene which comprises Site B, in order to perform site-directed mutagenesis as described below in Examples 1 and 2, using RARE cleavage. In brief, RecA-assisted restriction endonuclease (RARE) cleavage consists of protecting a specific restriction endonuclease site with a complementary oligonucleotide. In the presence of RecA, a triplex DNA complex is formed that prevents methylation at the protected sites, for example *EcoRI*-35763 and *EcoRI*-41496 were protected by oligonucleotides (5' gaaaactcccacagcaagctaagtattctgaattcattcaattgggagagacaa gtttcac 3') (SEQ ID NO:22) and (5' cacaagtgaatatctggttaggatagaattctcccagtttcacaatgaaaacatc 3') (SEQ ID NO:23) respectively,

while unprotected sites are methylated by the corresponding methylase. After dissociation of the oligonucleotides, the protected sites can be cleaved with the restriction endonuclease which corresponds to the protected sites, for example EcoRI. All of the non-protected EcoRI site had been methylated and were thus not subject to cleavage by the restriction enzyme. The resulting fragment of the apo-B100 gene can then be ligated into a smaller vector which is appropriate for site-directed mutagenesis, e.g. pZErO. The site-directed mutagenesis process is then conducted by techniques well known in the art, and the fragment is return and ligated to the larger vector from which it was cleaved. For site directed mutagenesis methods see, for example, Kunkel, T. 1985. *Proc. Natl. Acad. Sci. U.S.A.* 82:488; Bandeyar, M. et al. 1988. *Gene* 65: 129-133; Nelson, M., and M. McClelland 1992. *Methods Enzymol.* 216:279-303; Weiner, M. 1994. *Gene* 151: 119-123; Costa, G. and M. Weiner. 1994. *Nucleic Acids Res.* 22: 2423; Hu, G. 1993. *DNA and Cell Biology* 12:763-770; and Deng, W. and J. Nickoff. 1992. *Anal. Biochem.* 200:81.

Please replace the paragraph that begins at page 43, line 15, with the following paragraph:

The 95-kb apo-B P1 plasmid p158 (Linton, M. et al. 1993. *J. Clin. Invest.* 92:3029-3037) was prepared and modified by RARE cleavage as described by Borén, J. et al. 1996. *Genome Res.* 6:1123-1130. Oligomers *EcoRI*-35763 (5' gaaaactccacagcaagctaagtattatctgaattcattcaattgggagagacaagtttcac 3') (SEQ ID NO:22) and *EcoRI*-41496 (5' cacaagtgaaatatctggttaggtagaattctcccagtttcacaatgaaaacatc 3') (SEQ ID NO:23) were used to make 5.7-kb-deleted P1 plasmid. A 5.7-kb fragment was isolated from the apo-B100 "Leu-Leu" plasmid with RARE cleavage using oligomers *EcoRI*-35763 and *EcoRI*-41496 and cloned into the pZErO-1 vector (Invitrogen). The apo-B100 "Leu-Leu" plasmid was used to increase the yield of apo-B100, since it contains a CAA to CTA mutation in codon 2153 that effectively abolished the formation of apo-B48. The latter of which is formed by an editing mechanism present in mouse livers (Yao, Z. et al. 1992. *J. Biol Chem.* 267:1175-1182).

Please replace the paragraph that begins at page 44, line 3, with the following paragraph:

The pZER0-5.7 kb plasmid was subjected to site-directed mutagenesis with the Morph System (5 Prime→3 Prime, Inc.[®]) using oligonucleotide K3363E (5' caagattgacaagagaaaggggattgaag 3') (SEQ ID NO:24) to mutate the lysine at residue 3363 to glutamic acid. The resulting plasmids were subjected to RARE cleavage with oligomers *EcoRI*-35763 and *EcoRI*-41496, and the mutated 5.7-kb fragment was isolated. After RARE cleavage of the 5.7-kb-deleted P1 plasmid with oligonucleotide *EcoRI* del. 5.7-kb (5' ggaaaactcccacagcaagctaattgattatctgaattctccc agtttcacaatgaaaacatc 3') (SEQ ID NO:25), the mutated 5.7-kb fragment was ligated into the linearized and phosphatased 5.7-kb-deleted P1 vector. (Borén, J. et al. 1996. *Genome Res.* 6:1123-1130).